

## THE EFFECT OF X-RADIATION ON ENZYME SYSTEMS OF TETRAHYMENA PYRIFORMIS<sup>1, 2</sup>

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It had been shown previously by Eichel and Roth (1953b) that x-radiation at a level of 300,000 or 500,000 r depressed the respiration of *Tetrahymena pyriformis* W,<sup>3</sup> but did not appreciably affect the nuclease activity in homogenates prepared from irradiated whole cells. In irradiated homogenates, however, nuclease activity was reduced by approximately 50%. This paper presents some further observations of the effect of 300,000 to 600,000 r on respiration and several enzymes of *T. pyriformis* S. The S strain was chosen for two reasons; first, to obtain comparisons of the effect of x-radiation on respiration and deoxyribonuclease (DNase) activity in a strain different from that studied previously, and second, because the higher rate of succinate oxidation in the S strain (Eichel, 1954) would facilitate investigations of this specific enzyme system. The effects of x-radiation on catalase, and malic, glutamic, and succinic dehydrogenases, as well as some properties of succinic dehydrogenase and DNase, have also been observed. In addition, the effect of x-radiation on the oxidation of L-phenylalanine and acetate by the W strain has been studied.

### MATERIALS AND METHODS

*T. pyriformis* S cells were grown and harvested as previously described (Eichel and Roth, 1953b), and homogenates were prepared as described by Eichel (1954). Measurements of respiration and oxidative enzyme activities were by conventional Warburg techniques; the details of the procedures for x-irradiation and for the respiration and DNase studies were similar to those previously reported (Eichel and Roth, 1953b). Measurements of DNase activity were made at regular intervals for at least 100 to 136 minutes. The components of the test systems used in the study of oxidative enzyme activities are given in connection with the tables of results. All Warburg runs were made at 27° with air as the gas phase and a fluid volume of 3.0 ml. Catalase was determined by the method of Bonnicksen, Chance and Theörell (1947). Unless otherwise stated, 72 hour cultures of strain S were utilized for all experiments.

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<sup>2</sup> A preliminary account of this project was presented at the General Scientific Meetings, Marine Biological Laboratory, Woods Hole, Mass., in August 1953 (Eichel and Roth, 1953a).

<sup>3</sup> *T. pyriformis* was formerly called *T. geleii*.

## EXPERIMENTAL RESULTS

*The effect of x-radiation on respiration*

Concentrated cell suspensions (approximately  $2 \times 10^6$  cells/ml.), and cell suspensions diluted with an equal volume of glass-distilled water (referred to as diluted cell suspensions), were irradiated. The appearance of the irradiated cells immediately after exposure was similar to that of the x-irradiated W strain; many cells were non-motile, swollen, and highly vacuolated. The effect on respiration was comparable to that observed with the W strain (Eichel and Roth, 1953b) with the exception that the S organisms appeared more resistant at the 300,000 r level. It is evident from the data in Table I that 600,000 r was considerably more damaging than 300,000 r, and that at the higher level there was little difference in

TABLE I  
*Effect of x-irradiation with 300,000 and 600,000 r on respiration of T. pyriformis S*  
*The figures give the average and range*

Conditions	Per cent change in O <sub>2</sub> uptake from control cells			O <sub>2</sub> of control cells <sup>2</sup> ( $\mu$ l O <sub>2</sub> per hr. per mg. of dry wt.)
	Minutes after irradiation <sup>1</sup>			
	30	80	140	
Concentrated cell suspension (7) <sup>3</sup>	+12	-3	-9 <sup>4</sup>	32.3
300,000 r	(+3 to +17)	(0.0 to -7)	(-6 to -13)	(30.8 to 39.8)
Diluted cell suspension (6)	-4	-17	-23	29.9
300,000 r	(+40 to -43)	(0.0 to -47)	(-8 to -48)	(25.3 to 34.0)
Concentrated cell suspension (3)	-16	-40	-50	27.7
600,000 r	(+8 to -40)	(-22 to -52)	(-37 to -60)	(21.1 to 32.0)
Diluted cell suspension (6)	-5	-40	-54	27.8
600,000 r	(+35 to -38)	(-20 to -55)	(-40 to -70)	(26.7 to 29.5)

## Part 2. Effect of 2,6-diaminopurine (AP)

Diluted cell suspension +AP <sup>5</sup> (2) <sup>6</sup>	+13	-24	-41	
300,000 r	(0, +26)	(-17, -31)	(-36, -46)	
Diluted cell suspension (2) <sup>7</sup>	+31	-3	-9	
300,000 r	(+22, +40)	(0, -5)	(-8, -10)	
Diluted cell suspension +AP <sup>5</sup> (5) <sup>6</sup>	+23	-25	-41	
600,000 r	(+60 to -4)	(-9 to -45)	(-26 to -60)	
Diluted cell suspension (4) <sup>7</sup>	-8	-41	-57	
600,000 r	(+35 to -22)	(-20 to -48)	(-37 to -63)	

<sup>1</sup> The elapsed time between the end of irradiation and closing of the stopcocks was 20 minutes. Therefore, the first 10 minute reading was actually made 30 minutes after irradiation. In general, readings were made at 10-minute intervals during the first hour and at the end of 2 hours. For the sake of brevity, portions of these data are presented here.

<sup>2</sup> Calculated for the first hour.

<sup>3</sup> Numbers in parentheses indicate the number of separate experiments in both Part 1 and Part 2.

<sup>4</sup> Six determinations; two at 110 minutes, four at 120 minutes.

<sup>5</sup> AP concentration was  $3.3 \times 10^{-2}$  M during irradiation and  $6 \times 10^{-3}$  M after addition of aliquots to vessels.

<sup>6</sup> Control was unirradiated diluted cell suspension +AP.

<sup>7</sup> Control was unirradiated diluted cell suspension.

effect using concentrated or diluted cell suspensions. At 300,000 r, however, the concentrated cell suspensions showed less depression of respiration than those that were diluted. The results at 30 minutes after exposure to the x-ray beam were extremely variable except with concentrated suspensions irradiated with 300,000 r. The initial increase in respiration under these conditions is of interest; it is probably somewhat greater than the data indicate since staining (dilute methyl green) of the irradiated cells showed that many of them (25–50%) were dead immediately after irradiation.<sup>4</sup> In general, there was a significant decrease in respiration of the x-irradiated cells with time; this may be due to a continuing mortality of damaged organisms. The  $QO_2$  values of control cells are presented in Table I to indicate the level of activity in these experiments.

A general interest in the purine antagonist, 2,6-diaminopurine (AP), led us to study the effect on respiration of the presence of this compound during irradiation of whole cells. Controls consisted of unirradiated cells in the presence of similar concentrations of the antagonist. The results obtained with AP are given in Table I, part 2. It can be seen that this compound afforded appreciable protection at the higher level of exposure, particularly in the early post-irradiation period. On the other hand, at 300,000 r AP had a significant deleterious effect. The concentration of AP used (0.006 *M*) was slightly inhibitory to the respiration of control cells. At the present time it is not possible for us to offer an explanation of the effects observed. With regard to the protective action, several amino compounds have been shown to protect other x-irradiated animals (Bacq and Herve, 1951; Bacq *et al.*, 1953), and it is possible that AP is another member of this class.

#### *The effect of x-radiation on the oxidation of L-phenylalanine and acetate*

Of the common amino acids, *L*-phenylalanine is most rapidly oxidized by *T. pyriformis* W (Roth, Eichel and Ginter, 1954). The rate of oxidation of acetate is also high in this organism (Kidder and Dewey, 1949). In view of the stimulatory effect of these compounds on the respiration of whole cells, it was deemed of interest to determine what effect they would have when added to x-irradiated cells. Strain W was used since it was investigated previously with respect to the oxidation of these metabolites. Diluted cell suspensions were prepared and then irradiated with 300,000 r. Immediately afterward, aliquots of the suspensions were added to Warburg vessels whose sidearms contained either neutral *L*-phenylalanine or acetate solution. Oxygen consumption was measured after tipping the sidearms and was compared to unirradiated controls to which the same additions had been made, as well as to controls, both irradiated and non-irradiated, to which only distilled water had been added from the sidearms. Experimental details and results are given in Table II. It can be seen that the oxygen consumption in the presence of *L*-phenylalanine was inhibited by 43% (average of 4 experiments) 30 minutes after irradiation. This inhibition was observed to increase at a uniform rate until, at 140 minutes post-irradiation, the value was 58%. The decrease in oxygen uptake of irradiated cells to which phenylalanine had been added was somewhat greater than that observed for the irradiated cells without phenylalanine, studied at the same time. The conclusion is that x-irradiation impairs the ability

<sup>4</sup> Living cells, even those visibly severely damaged, do not take up the stain; dead cells stain a light green with a darker green nucleus.

TABLE II

Effect of *x*-irradiation with 300,000 r on oxidation of *L*-phenylalanine and acetate by *T. pyriformis*. Each vessel contained the following: body, 1.0 ml. of either control or irradiated cells and 1.0 ml. of 0.1 M  $\text{Na}_2\text{HPO}_4\text{-NaH}_2\text{PO}_4$  buffer, pH 7.3; sidearm, substrate as indicated below or water; center well, 0.2 ml. of 20% KOH. The vessels were allowed to equilibrate 5 minutes before tipping in substrate. After an additional 5 minutes, the stopcocks were closed and readings were taken at 10-minute intervals during the first hour and at the end of 2 hours

Additions from sidearm	Conditions and strain	O <sub>2</sub> uptake in $\mu\text{l}$ .					
		Minutes after closing stopcocks <sup>1</sup>					
		10	% change	60	% change	120	% change
<i>L</i> -phenylalanine <sup>2</sup> <i>L</i> -phenylalanine	(unirradiated) W(5) <sup>3</sup> (irradiated)	31		202		430	
		24	-23	146	-28	249	-42
Acetate <sup>4</sup> Acetate	(unirradiated) (irradiated)	43		270			
		51	+19	250	-7		
Water Water	(unirradiated) (irradiated)	30		182		354	
		26	-13	143	-21	251	-29
<i>L</i> -phenylalanine <i>L</i> -phenylalanine	(unirradiated) S(3) (irradiated)	29		179		336	
		15	-48	86	-52	142	-58
Acetate Acetate	(unirradiated) (irradiated)	31		177		337	
		36	+16	167	-6	260	-23
Water Water	(unirradiated) (irradiated)	32		157		275	
		18	-43	84	-47	131	-48
<i>L</i> -phenylalanine <i>L</i> -phenylalanine	(unirradiated) W(2) (irradiated)	17		148		301	
		9	-47	64	-57	101	-66
Acetate Acetate	(unirradiated) (irradiated)	25		158		283	
		20	-20	125	-21	170	-40
Water Water	(unirradiated) (irradiated)	14		104		203	
		6	-57	47	-55	81	-60
<i>L</i> -phenylalanine <i>L</i> -phenylalanine	(unirradiated) W(2) (irradiated)	29		188		356	
		14	-52	80	-57	129	-64
Acetate Acetate	(unirradiated) (irradiated)	35		184		341	
		33	-6	153	-17	227	-33
Water Water	(unirradiated) (irradiated)	21		125		240	
		16	-24	89	-29	154	-36
Averages							
<i>L</i> -phenylalanine			-43		-49		-58
Acetate			+ 2		-13		-32
Water			-35		-38		-43

<sup>1</sup> See note 1, Table I.<sup>2</sup> Final concentration =  $3 \times 10^{-3}$  M.<sup>3</sup> Numbers in parentheses indicate age of culture in days.<sup>4</sup> Final concentration =  $5 \times 10^{-2}$  M.

of the cells to oxidize *L*-phenylalanine. In several cases acetate stimulated oxygen consumption by irradiated cells at 30 minutes after exposure; at 80 minutes after exposure, oxygen uptake was inhibited by only 13% as compared to the 38% inhibition of endogenous respiration. Although the inhibition of acetate oxidation increased considerably between 30 and 140 minutes after irradiation, the final level attained was less than that of either the endogenous respiration or phenylalanine oxidation.

The marked ability of acetate to stimulate the oxygen uptake of irradiated cells is of considerable interest. For example, in the third experiment at 80 minutes after exposure, irradiated cells to which acetate had been added showed an increase of 170% in oxygen uptake compared to irradiated cells without acetate. In the same experiment, acetate increased the oxygen consumption of control cells

TABLE III

*Succinic dehydrogenase activity of x-irradiated homogenates and of homogenates prepared from x-irradiated cells of T. pyriformis S. Each vessel contained the following: body, 1.0 ml. of either control or irradiated homogenate, 1.0 ml. of 0.1 M Na<sub>2</sub>HPO<sub>4</sub>-NaH<sub>2</sub>PO<sub>4</sub> buffer, pH 7.3, 0.3 ml. of 0.004 M CaCl<sub>2</sub> + AlCl<sub>3</sub>, and 0.2 ml. of distilled water; sidearm, 0.3 ml. of 0.5 M sodium succinate adjusted to pH 7.4; center well, 0.2 ml. of 20% KOH. Since it has been shown that under the conditions employed here added cytochrome c has little effect on the activity of this system (Eichel, 1954), the compound was omitted. Conditions of equilibration and reading the same as those described in Table II. The figures give the average and range*

Conditions	Dose	Per cent change in O <sub>2</sub> uptake from control homogenates		
		Minutes after irradiation		
	(r)	30	80	140
Irradiated homogenates	300,000 (4)*	-28 (+5 to -45)	-34 (-14 to -48)	-27 (-4 to -50)
	600,000 (4)		-41 (-30 to -53)	-38 (-24 to -49)
Homogenates of irradiated cells	300,000 (4)	-15 (0 to -25)	-10 (-7 to -15)	-6 (+4 to -12)

\* Numbers in parentheses indicate the number of separate experiments.

by only 52%. The net effect of the increased oxidation of acetate by irradiated cells is to give the appearance of a protective action by acetate, but in this case the acetate has been added after irradiation and the only protection would be that related to delayed effects of radiation. While certain unknown and probably complex factors will protect animals when given after irradiation (Kelly and Jones, 1953; Cole and Ellis, 1954), no simple substances have yet been shown to have this effect, and it may be worthwhile to test acetate for this action in higher animals.

#### *The effect of x-radiation on respiratory enzymes*

##### *Succinic dehydrogenase*

Homogenates were irradiated; also, whole cells were irradiated and then homogenized. In all cases suitable unexposed controls were prepared and as-

sayed simultaneously with the irradiated samples. The contents of the Warburg vessels and other experimental details are presented in Table III which gives the results obtained. Irradiation of homogenates at 300,000 or 600,000 r resulted in the loss of a considerable fraction of the activity. However, the activity of homogenates prepared from cells irradiated at 300,000 r declined only slightly, the decrease being just on the borderline of significance.<sup>5</sup>

Seaman (1952), using cystine as an inhibitor, concluded that *Tetrahymena* succinic dehydrogenase is a sulfhydryl-dependent enzyme. In Table IV are listed some effects of SH-reagents on the succinic dehydrogenase activity of normal homogenates. The strong inhibition of the system by *p*-chloromercuribenzoate (CMB) and *o*-iodosobenzoate (IOB) suggests further that it is SH-dependent, although the activity was not restored by the addition of glutathione at concentrations 10–20 times those of the inhibitors. The glutathione was either present in the main body of the vessels at the start of the experiment or tipped in from the sidearm midway through the run. The very slight reduction in succinic dehydro-

TABLE IV

*Effect of p-chloromercuribenzoate (CMB) and o-iodosobenzoate (IOB) on succinic dehydrogenase activity of homogenates of T. pyriformis S. Assays were performed as described in Table III except that in one experiment succinate was added to the body instead of the sidearm together with the sulfhydryl reagents. Concentrations given for the latter are final. The figures represent the average of 2 experiments*

Addition	Per cent inhibition
$0.9 \times 10^{-3} M$ CMB	–98
$0.1 \times 10^{-3} M$ CMB	–84
$0.1 \times 10^{-2} M$ IOB	–94
$0.5 \times 10^{-3} M$ IOB	–83

genase activity resulting from the irradiation of whole cells is of interest in view of the studies of Barron (1946) which indicated that inactivation of SH-enzymes is an important aspect of radiation damage in animal tissues. Previously, it had been shown that the activity of the SH-dependent ribonuclease of *T. pyriformis* W was relatively unchanged after exposing cells to very high doses of x-rays (Eichel and Roth, 1953b). These observations with protozoan enzymes are consistent with the recent reports that the activities of SH-dependent enzymes are frequently not altered in the tissues of mammals exposed to high levels of x- or  $\gamma$ -radiation (LeMay, 1951; Roth *et al.*, 1952; Thomson *et al.*, 1952; Ashwell and Hickman, 1952; Roth *et al.*, 1953).

#### *Glutamic and malic dehydrogenases*

The conditions and results are given in Table V. The activities of both enzymes were significantly reduced at 80 minutes; after an additional hour, the inhibition of glutamic dehydrogenase was apparently decreased while there was little further change in malic dehydrogenase.

<sup>5</sup> Changes of less than 10% are not considered significant.

TABLE V

*Glutamic and malic dehydrogenase activities of T. pyriformis S homogenates x-irradiated with 600,000 r. Both enzymes were assayed by the method of Seaman (1951) except that each vessel contained 0.5 ml. of 0.1 M Na<sub>2</sub>HPO<sub>4</sub>-NaH<sub>2</sub>PO<sub>4</sub> buffer, pH 7.3. Conditions of equilibration and reading the same as those described in Table II. The figures give the average and range*

Dehydrogenase	Per cent change in O <sub>2</sub> uptake from control homogenates	
	Minutes after irradiation	
	80	140
Glutamic (3)*	-28 (-14 to -40)	-10 (-9 to -10)
Malic (4)	-40 (-9 to -65)	-41 (-21 to -60)

\* Numbers in parentheses indicate the number of separate experiments.

### Catalase

*T. pyriformis S* contains an active and, under the conditions employed here, relatively unstable catalase which has not been previously described. No loss of enzyme activity was observed in homogenates kept at 0° up to 2½ hours. However, after 24 hours at this temperature, the activity decreased by 50%. In preliminary experiments, the activity was found to be proportional to the quantity of homogenate used. Catalase was significantly inhibited in x-rayed homogenates tested 5 minutes after irradiation (Table VI), but the activity was rapidly recovered when these homogenates were allowed to stand at 0° in the absence of substrate. In two experiments in which homogenates were irradiated at 600,000 r (not tabulated), and in which catalase assays were performed at 5, 35, 65, 95 and 155 minutes after irradiation, the average changes from control values were -36, -9, -10, -1 and +2%, respectively. After 24 hours, the irradiated homogenates were 29% more active than the controls. A similar recovery of activity was observed in homogenates prepared from irradiated cells (not tabulated). In one experiment with the latter, the catalase activity was 29% less than the control 10 minutes after irradiation, while 120 minutes later, it was 10% more active than the unchanged control.

TABLE VI

*Catalase activity of x-irradiated homogenates of T. pyriformis S. Each determination performed with 1.0 ml. of homogenate. Measurements made within 5 minutes after completion of irradiation*

Dose (r)	Specific activity × 1000		Change (%)
	Control (per sec. per mg. dry wt.)	X-ray (per sec. per mg. dry wt.)	
300,000 (2)*	2.14 (1.99, 2.29)	1.85 (1.99, 1.72)	-13 (0, -25)
600,000 (5)	2.36 (2.10 to 2.77)	1.67 (1.12 to 2.27)	-29 (-11 to -50)

\* Numbers in parentheses indicate the number of separate experiments.

TABLE VII

Effect of *x*-irradiation with 500,000 r, and effect of sulfhydryl reagents, on DNase activity of homogenates of *T. pyriformis* S. In part 1, experiments were performed as previously described for ribonuclease (Eichel and Roth, pp. 355-56, 1953b). Homogenates were irradiated in the presence of *p*-chloromercuribenzoate and aliquots were used for DNase assays. Before assaying, *p*-chloromercuribenzoate, in the concentration listed below, was in contact with the homogenate for 100 minutes, the time required for the irradiation procedure. The addition of aliquots of these samples to the assay mixtures diluted the *p*-chloromercuribenzoate to  $3.5 \times 10^{-4}$  M. The cysteine was added after irradiation in a final concentration of  $3.3 \times 10^{-3}$  M. In parts 2 and 3, the final concentrations are listed. The figures give the average and range of three experiments in part 1 and two experiments each in parts 2 and 3

Part	Experimental conditions	Specific activity $\times 100$ (units <sup>1</sup> per mg. dry wt.)	Change (%)
1	Control	1.38 (1.22 to 1.60)	
	Irradiated	0.66 (0.46 to 0.90)	-57 (-44 to -72)
	Control + $2.8 \times 10^{-3}$ M CMB	1.20 (0.86 to 1.47)	-15 (+11 to -30)
	Irradiated + $2.8 \times 10^{-3}$ M CMB	0.19 (0.13 to 0.29)	-86 (-78 to -94)
	Irradiated + $2.8 \times 10^{-3}$ M CMB + $3.3 \times 10^{-3}$ M L-cysteine	0.27 (0.07 to 0.55)	-82 (-58 to -99)
2	Control	1.23 (1.09, 1.36)	
	Control + $2.2 \times 10^{-4}$ M CMB	1.09 (1.02, 1.16)	-8 (-6, -10)
	Control + $1.7 \times 10^{-3}$ M L-cysteine	0.84 (0.79, 0.89)	-30 (-28, -31)
	Control + $2.2 \times 10^{-4}$ M CMB + $1.7 \times 10^{-3}$ M L-cysteine	1.04 (0.98, 1.11)	-11 (-8, -14)
	Control + $1.1 \times 10^{-3}$ M IOB <sup>2</sup>	1.28 (1.13, 1.44)	+8 (+4, +11)
3	Control	0.76 (0.80, 0.72)	
	Control + $5.6 \times 10^{-5}$ M CMB	0.87 (1.00, 0.74)	+19 (+26, +3)
	Control + $2.8 \times 10^{-4}$ M CMB	0.73 (0.84, 0.63)	-4 (+6, -13)
	Control + $5.6 \times 10^{-4}$ M CMB	0.72 (0.72, 0.72)	-5 (-10, 0)
	Control + $1.1 \times 10^{-3}$ M CMB	0.34 (0.32, 0.35)	-55 (-60, -51)

<sup>1</sup> One unit of activity is defined as that amount of enzyme which, in 25 ml. of test solution, causes a decrease in optical density of 0.1 in 60 minutes.

<sup>2</sup> *o*-Iodosobenzoate.

#### *The effect of x-radiation and sulfhydryl reagents on DNase activity*

It has been reported previously that DNase activity decreased 50% in *T. pyriformis* W homogenates irradiated at 500,000 r (Eichel and Roth, 1953b). Similar results were obtained with the S strain and these are given in Table VII, part 1. The DNase activity of control homogenates was of the same magnitude as that found previously for the W strain. The effect of CMB, both alone and in com-



bination with x-radiation, was also investigated. The combined inhibitory effects of radiation and the presence of CMB were somewhat more than additive, while the addition of cysteine after irradiation had little effect on the inhibition. In a similar experiment with ribonuclease of the W strain, cysteine added to irradiated homogenates completely reversed the inhibition due to CMB (Eichel and Roth, 1953b). While cysteine (Table VII, part 2) inhibited DNase by 30% and CMB depressed the activity by 8%, together the two compounds produced only an 11% inhibition. CMB alone had a variable effect depending on its concentration (Table VII, part 3). At the highest concentration used, DNase activity was depressed strongly, while at the lowest concentration there was a slight acceleration. This variable response to CMB has also been reported with pancreatic ribonuclease (Ledoux, 1953). IOB accelerated DNase activity slightly. These facts suggest that in *T. pyriformis* S DNase is dependent for optimum activity on a certain ratio of -S-S- to -SH groups.

#### DISCUSSION

From the results reported in this paper and the previous one (Eichel and Roth, 1953b) it seems clear that irradiation of *Tetrahymena* homogenates at 300,000 to 600,000 r results in appreciable destruction of enzyme activity. However, in the case of catalase, where irradiated homogenates were allowed to stand at 0° and assayed at varying intervals, all of the activity was recovered within 1-2 hours after x-raying. On the other hand, the irradiation of whole cell suspensions, while causing considerable decrease in respiration, does not markedly affect the activity of several enzymes tested. It seems probable, therefore, that the explanation of the high cell mortality observed does not lie in alterations in enzyme activity due to the direct or indirect action of radiation. Other factors, such as denaturation of nucleoprotein resulting in lethal mutations, or formation of toxic products *in vivo*, may be involved. Further biochemical studies on radiation effects might be more profitably directed toward changes in nuclear activities, but before this can be done we must learn more about the fundamental properties of the nucleus.

#### SUMMARY

1. The effect of 300,000 to 600,000 r on respiration and oxidative and other enzymes of *Tetrahymena pyriformis* S has been determined. Both homogenates and whole cells were irradiated. At 600,000 r, respiration was significantly decreased; the activities of the succinic, glutamic, and malic dehydrogenase systems, catalase, and DNase were depressed in homogenates irradiated at this level. Within a short time after irradiation, catalase activity was completely recovered in homogenates allowed to stand at 0°. Irradiated cells showed only minor changes in succinic dehydrogenase activity.

2. Whole cells irradiated with 600,000 r in the presence of 2,6-diaminopurine were considerably protected from the effects of radiation on respiration for a short time after exposure. Cells of the W strain, irradiated with 300,000 r and then placed in the presence of *L*-phenylalanine, showed a loss of ability to oxidize this compound compared to controls. Under the same conditions acetate stimulated the oxygen consumption of x-irradiated cells to a much greater degree than that of control cells when measured 30 minutes after irradiation. Some effects of

sulfhydryl reactants on succinic dehydrogenase and deoxyribonuclease activities of homogenates were studied, and the general implications of the results were discussed.

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